

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N Engl J Med. DOI: 10.1056/NEJMc2016359

# Supplemental appendix

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## Methods

### *Ethics*

All study participants were enrolled and sampled in accordance with the Yale University HIC-approved protocol #2000027690. Demographics, clinical data and samples were collected after the study participant had acknowledged that they had understood the study protocol and signed the informed consent. All participant information and samples were collected in association with non-individually identifiable study identifiers.

## ***Participant enrollment***

### Inpatients

Patients admitted to Yale-New Haven Hospital (a 1541-bed tertiary care medical center in New Haven, CT, USA), who tested positive for SARS-CoV-2 by nasopharyngeal and/or oropharyngeal swab with CLIA-approved assay performed by the hospital were invited to enroll in the research study. Exclusion criteria were non-English speaking and clinical, radiological or laboratory evidence for a non-infectious cause of fever or respiratory symptoms or a microbiologically-confirmed infectious source (e.g. gastrointestinal, urinary, cardiovascular) other than respiratory tract for symptoms and no suspicion for COVID-19 infection.

### Healthcare workers

Asymptomatic healthcare workers (e.g., without fever or respiratory symptoms) working on COVID-19 units and infirmaries or with occupational exposure to patients with COVID-19 were invited to enroll into a prospective study that implemented an active monitoring protocol for SARS-CoV-2 infection for the purpose of ensuring early detection following exposure and protecting other healthcare workers and patients. Exclusion criteria were age under 18 years and non-English speaking.

## ***Study population***

Between March 23rd and June 16th, 2020, 321 COVID-19 inpatients at Yale-New Haven Hospital were identified as being eligible to enroll in our study and were approached by the clinical team. Of these, 202 individuals were enrolled.

In addition, 495 asymptomatic healthcare workers (aged 22-74 years, average = 37.6 years; male, n = 105, 21%) were also enrolled into the active monitoring protocol.

## ***Sample collection***

### Inpatients

The collection of nasopharyngeal and saliva samples was attempted every three days throughout their clinical course. Nasopharyngeal samples were taken by registered nurses using the BD universal viral transport (UVT) system. The flexible, mini-tip swab was passed through the patient's nostril until the posterior nasopharynx was reached, left in place for several seconds to absorb secretions then slowly removed while rotating. The swab was

placed in the sterile viral transport media (total volume 3 mL) and sealed securely. Saliva samples were self-collected by the patient using the methods described.<sup>1</sup> Upon waking, patients were asked to avoid food, water and brushing of teeth until the sample was collected. Patients were asked to repeatedly spit into a sterile urine cup until roughly a third full of liquid (excluding bubbles), before securely closing it. All samples were stored at room temperature and transported to the research lab at the Yale School of Public Health within 5 hours of sample collection and tested within 12 hours of sample collection. While a stabilizing solution was not added to the saliva, recent work has demonstrated that SARS-CoV-2 RNA is stable in saliva stored at room temperature for up to 25 days.<sup>2,3</sup>

#### Healthcare workers

Healthcare workers were asked to collect a self-administered nasopharyngeal swab and a saliva sample every three days for up to 84 days, or until testing positive for SARS-CoV-2. Samples were stored at +4°C until being transported to the research lab.

#### ***SARS-CoV-2 detection***

On arrival at the research lab, total nucleic acid was extracted from 300 µl of viral transport media from the nasopharyngeal swab or 300 µl of whole saliva using the MagMAX Viral/Pathogen Nucleic Acid Isolation kit (ThermoFisher Scientific) with a modified protocol.<sup>1</sup> If a saliva sample proved difficult to pipette due to high viscosity, 10 µl of Proteinase K was added to the original sample and then vortexed vigorously for 30 seconds to liquify the sample, before proceeding with extraction. While unusual for asymptomatic healthcare worker saliva this was more common for the COVID-19 inpatient saliva samples received, likely due to the first morning sample collecting the accumulation of overnight secretions.

Following extraction, RNA was eluted into 75 µl of elution buffer. For SARS-CoV-2 RNA detection, 5 µl of eluted RNA template was tested as previously described,<sup>4,5</sup> using the US CDC real-time RT-qPCR primer/probe sets for 2019-nCoV\_N1 and 2019-nCoV\_N2 and the human *RNase P* (RP) as an extraction control. Samples were classified as positive for SARS-CoV-2 when both N1 and N2 primer-probe sets were detected <38 C<sub>T</sub>. Virus RNA copies were quantified per milliliter of clinical sample input volume (whole saliva for viral transport media) using the formula  $(10^{((Ct-43.023)/-3.718)}) \times 75 \times (1000/300)$  based off a 10-fold dilution standard curve of RNA transcripts that we previously generated.<sup>5</sup> As such, the lower limit of detection is

5,610 virus RNA copies/mL of sample, or 22 virus RNA copies/μl of RNA. As results from N1 and N2 were comparable (**Fig. S4**), all virus copies are shown as calculated using the N1 primer-probe set.

In the event that a sample collected from an asymptomatic healthcare worker tested positive, the study team was notified. Individuals testing positive were referred for an FDA EUA test in a CLIA-certified diagnostic lab.

### **Statistical analysis**

Further statistical analyses were conducted in GraphPad Prism 8.0.0, described as follows and in the figure legends. We compared the analytical sensitivity of SARS-CoV-2 RNA detection between saliva and nasopharyngeal swabs from COVID-19 inpatients using a Wilcoxon signed-rank test of the virus RNA copies and reported the mean and 95% confidence interval from log-transformed the data. We evaluated test positivity for the first available paired saliva and nasopharyngeal samples from the 70 inpatients collected after their COVID-19 diagnosis.

We also evaluated the kinetics of SARS-CoV-2 RNA titers during hospitalization as a function of time from onset of symptoms. We analyzed the data using a linear mixed effects regression model that accounts for the correlation between samples collected from the same person at a single time period (i.e., multivariate response) and the correlation between samples collected across time from the same patient (i.e., repeated measures). The model for people with repeated samples is given as

$$Y_{ijk} = \beta_{0j} + \beta_{1j}x_{ik} + \theta_{ij} + \eta_{ik} + \epsilon_{ijk}$$

while the model for people with a single sample is given as

$$Y_{ijk} = \beta_{0j} + \beta_{1j}x_{ik} + \eta_{ik} + \delta_{ijk}$$

where  $Y_{ijk}$  is the number of SARS-CoV-2 copies/mL for person  $i$ , sample type  $j$  ( $j=1$ : nasopharyngeal;  $j=2$ : saliva), and replicate  $k$  ( $k=1, \dots, m_i$ ) and  $x_{ik}$  is the number of days since symptom onset for person  $i$  at replicate  $k$ . Prior to analysis, we  $\log_{10}$  transformed the data, after adding one to all observations to achieve approximate normality of the residuals.

The random effects are defined as

$$\theta_{ij} \stackrel{\text{iid}}{\sim} N(0, \sigma_{\theta_j}^2) \text{ and } \eta_{ik} \stackrel{\text{iid}}{\sim} N(0, \sigma_{\eta}^2)$$

where we allow for separate variance parameters for the different sample types. The first set of effects account for correlation due to the repeated measures while the second set account for correlation due to the multivariate response. We also allow for separate error variance parameters for people with and without repeated measures, once again varying by sample type, such that

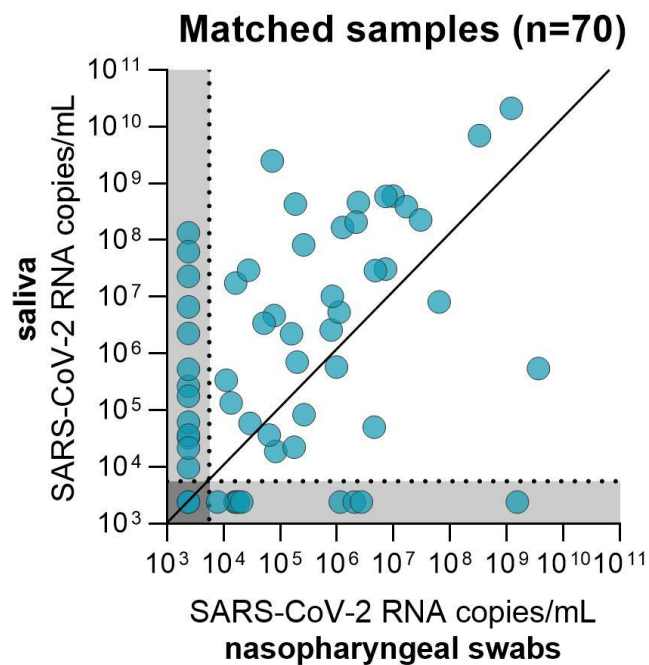
$$\epsilon_{ijk} \stackrel{\text{iid}}{\sim} N(0, \sigma_{\epsilon_j}^2) \text{ and } \delta_{ijk} \stackrel{\text{iid}}{\sim} N(0, \sigma_{\delta_j}^2).$$

Using output from this model, we are able to estimate the slopes for each sample type ( $\beta_{1j}$ ) and the variability of the observations around the estimated regression lines (for people with repeated measures) for each sample type ( $\sigma_{\epsilon_j}^2$ ).

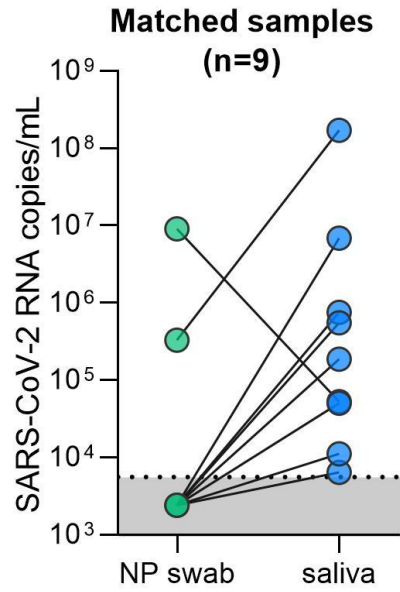
We fit the model in the Bayesian framework using weakly informative prior distributions. Specifically, the regression parameters are given Gaussian priors centered at zero with a standard deviation of 100 and the standard deviation parameters are given uniform priors with a lower bound of zero and upper bound of 1,000. We collect one million posterior samples after discarding the first 100 thousand during a burn-in period. We thin the samples by a factor of 100 to reduce posterior autocorrelation, resulting in 10 thousand nearly independent samples from the posterior. All models are fit using the `rjags` package.<sup>6</sup> Posterior means and 95% equal tailed quantile-based credible intervals are used to summarize the marginal posterior distributions of interest.

**Table S1. COVID-19 inpatient cohort characteristics**

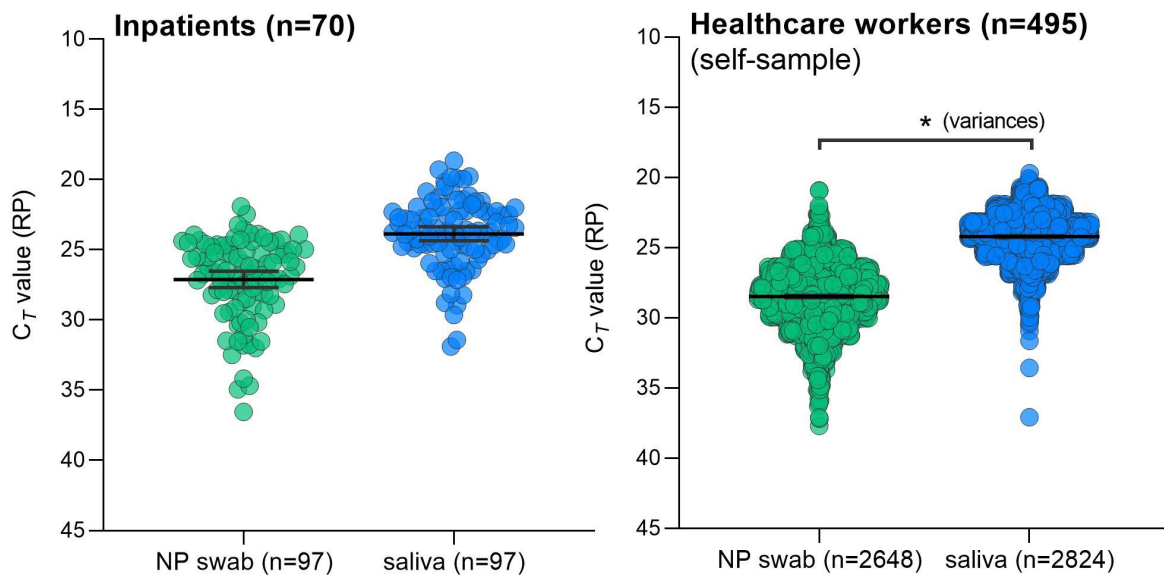
|   | Study participants ( $n = 70$ ) |
|---|---------------------------------|
| Gender, male                            | 41 (56%)                        |
| Age range, years                        | 13-91 (mean = 61.4)             |
| ICU on admission, $n$                   | 20 (29%)                        |
| ICU during hospital stay, $n$           | 35 (50%)                        |
| Mechanical ventilation, $n$             | 23 (33%)                        |
| Deceased (June 16 <sup>th</sup> ), $n$  | 10 (14%)                        |
| Discharged June 16 <sup>th</sup> ), $n$ | 59 (84%)                        |



**Fig. S1. SARS-CoV-2 RNA titers are higher in saliva than nasopharyngeal swabs from COVID-19 positive hospital inpatients.** SARS-CoV-2 RNA titers of the first available patient-matched nasopharyngeal and saliva samples ( $n = 70$ ), with higher virus RNA concentrations generally detected in matched saliva samples as compared to nasopharyngeal swabs (Wilcoxon signed-rank test ( $p < 0.001$ )).

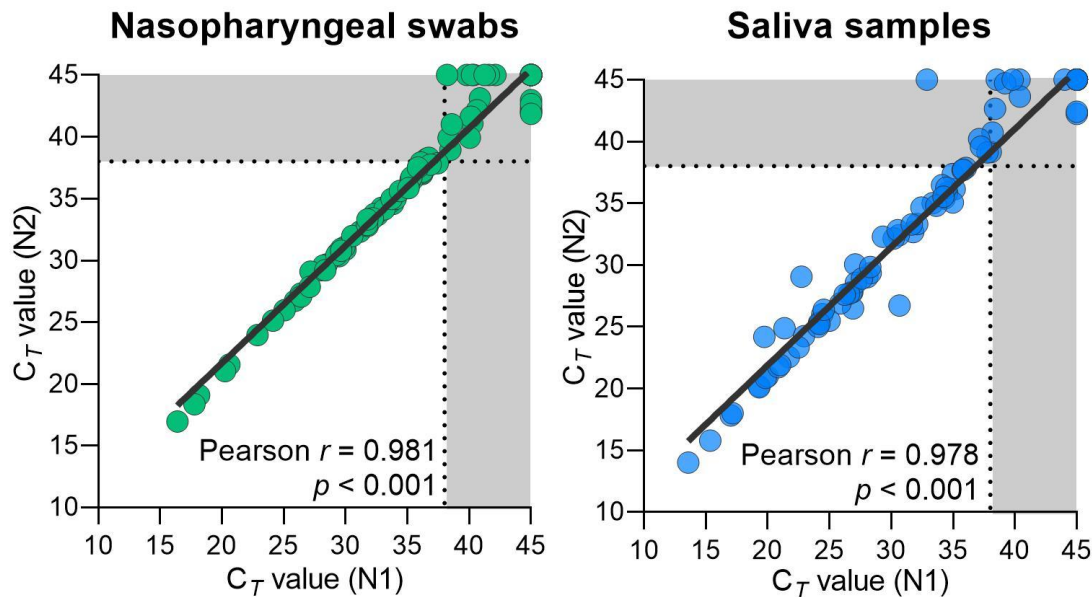


**Fig. S2. Saliva is an alternative for screening asymptomatic healthcare workers for SARS-CoV-2.** SARS-CoV-2 RNA copies measured from the 9 person-matched nasopharyngeal and saliva samples, collected from asymptomatic healthcare workers, in which at least one of the samples tested positive for SARS-CoV-2. Our assay detection limits for SARS-CoV-2 using the US CDC “N1” assay is at cycle threshold 38, which corresponds to 5,610 virus copies/mL of sample (shown as dotted line and grey area).



**Fig. S3. RT-qPCR detection of human *RNAse P* as a measure of sample quality shows greater variability in the quality of self-collected nasopharyngeal swabs as compared to saliva samples.**

RT-qPCR cycle thresholds (Ct) values for human *RNase P* (RP), an internal control for sample collection, from saliva and nasopharyngeal swabs from inpatients (left panel) and healthcare workers (right panel) were compared by variances using the F test ( $p = 0.15$  for inpatients;  $p < 0.001$  for healthcare workers). All of the data used to generate this figure, including the raw cycle thresholds, can be found in **Supplementary Data 1**.



**Fig. S4. Concordance between SARS-CoV-2 detection in 97 samples of each type using US CDC “N1” and “N2” primer and probe sets.** Ct = RT-qPCR cycle threshold. Dotted line and grey areas indicate the limits of detection.

## Acknowledgments

We gratefully acknowledge the study participants for their time and commitment to the study. We thank all members of the clinical team at Yale-New Haven Hospital for their dedication and work which made this study possible. We also thank S. Taylor and P. Jack for technical discussions. The study was partially funded by the Yale Institute for Global Health. The corresponding authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

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